PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
33484-00/PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/17020	20/06/2000	25/06/1999
International Patent Classification (IPC) or national classification and IPC		
C12N1/21		
Applicant		
AMERICAN CYANAMID COMPANY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/01/2001	18.09.2001
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Loubradou, G Telephone No. +49 89 2399 8543

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/17020

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-40 as originally filed

Claims, No.:

1-17 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

Sequence listing part of the description, pages:

1, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-7
	No:	Claims	1, 2, 8-17
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-17
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: WO 90 02557 A (PRAXIS BIOLOG INC) 22 March 1990 (1990-03-22)

D2: YANG Y -P ET AL: 'Effect of lipid modification on the physicochemical, structural, antigenic and immunoprotective properties of Haemophilus influenzae outer membrane protein P6' VACCINE,GB,BUTTERWORTH SCIENTIFIC. GUILDFORD, vol. 15, no. 9, 1 June 1997 (1997-06-01), pages 976-987, XP004115363 ISSN: 0264-410X cited in the application

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. D1 discloses a plasmid containing the lac promoter wherein said promoter is operatively linked to an isolated DNA sequence encoding the PBOMP-1 protein (PBOMP-1 is an other name for the P6 protein of the present application) (see page 81 lines 3 to 22). The possibility to use alternative regulated promoter is disclosed page 29 line 27 to page 30 line 23. The protein obtained using said construct is inherently in the lipidated form since it contains all the signals necessary for the lipidation of the protein.
Said plasmid was expressed in several E. coli strains including for example JM103 and HB101 strains (page 81 lines 23 to 25). Said strains were cultured in conditions which permit the expression of the lipidated recombinant PBOMP-1 protein.
Therefore D1 is prejudicial to the novelty of claims 1, 2 and 8 to 10.
The attention of the applicant is drawn to the fact that the use of the vague and unclear terms "tightly regulated promoter" leaves the reader in doubt as to the meaning of the technical features to which they refer. Such terms cannot be used to delimit the scope of claim 1 from the prior art (see also paragraph 4).

2.1 The attention of the applicant is drawn to the fact that a product is not rendered novel merely by the fact that it is produced by the way of a new process. Since, the lipidated recombinant P6 protein of the present application was not shown to

be different or to have different properties when compared to lipidated P6 purified from H. influenzae, antigenic compositions prepared with lipidated P6 purified from H. influenzae are considered prejudicial to the novelty of claims 11 to 13. There is many examples of such compositions in the prior art, see for example D2 (paragraph bridging pages 978 and 979, second full paragraph of the right hand column of page 979 and paragraph bridging pages 982-984). In said example aluminium phosphate is used is some cases as an adjuvant and several mammals (guinea pigs, mice and rabbits) were immunized. Therefore, D2 is prejudicial to the novelty of claims 11 to 17.

2.2 In addition, recombinant lipidated P6 protein are also known from the prior art. The production of recombinant lipidated P6 protein is disclosed in D1 section 6.8 pages 70 and 71. D1 also discloses the use of adjuvants including aluminium hydroxide and aluminium phosphate (see page 39 lines 18 to 33) and the immunisation of mammals (see page 37 lines 26 to 35 and page 38 lines 15 to 21). Therefore, D1 is also prejudicial to the novelty of claim 11 to 17.

3. The subject-matter of claims 3 to 7 appears to be novel with respect to the prior art cited in the International Search Report. The cited documents do not mention or suggest the use of a more "tightly regulated promoter" in order to solve the problem due to the instability of the lipidated P6 protein.
However, it is well known in the art of recombinant protein production that in the case of unstable protein inducible promoters with tight regulation should be used. In addition, the present application does not provide comparative examples with the plasmids of the closest prior art (see paragraphs 1. and 2. of the present Written Opinion) . Moreover, it is at present not clear whether the plasmid of the present application itself would be responsible for the increased amount of protein produced or whether the combination of specific plasmid/host cell is required (see page 7 line 30 to page 8 line 2). Therefore, at present claims 3 to 7 are not considered as involving an inventive step (Article 33.3 PCT).

4. Claims 16 and 17 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item VIII

Certain observations on the international application

5. As already mentioned in paragraph 1., the terms "tightly regulated promoter" used in claim 1 are vague and unclear and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT). This objection is of major relevance since the choice of the promoter appear to correspond to the core of the present application and since a prior art document with very close subject-matter, D1, exists.

6. The plasmid of claim 1 should not be defined in term of the function to be achieved, "wherein said DNA sequence, under the control of said promoter is expressed in lipidated form" (the applicant should also note that a DNA sequence cannot be expressed in lipidated form, only the protein encoded by said DNA can be expressed in lipidated form) (Article 6 PCT).
The same is also true for the antigenic composition of claim 11 "wherein said antigenic composition elicits a protective immune response in a mammalian host".

7. Unless the designation for a plasmid is internationally accepted (e.g. pUC18), a plasmid should not be identified in a claim by the use of an arbitrary designation. The plasmids "pPX4020" and "pPX4019" of claims 5 and 7 should therefore be characterised by technical features. Alternatively a deposit number could be given in the claims if such a basis exist in the application as originally filed.

8. The attention of the applicant is drawn to the fact that although no unity objections were at present raised (due to the lack of novelty of claims 11 to 17), there is no "special technical features" in the sense of Article 13.2 PCT linking the subject-matter of claims 1 to 10 and the subject matter of claims 13 to 17 since the subject-matter of claims 11 and 12 which formally links the two inventions, is clearly not novel (see paragraph 2.2).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
33484-00/PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 17020	20/06/2000	25/06/1999
Applicant		
AMERICAN CYANAMIC COMPANY		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/17020

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N1/21 C12N15/70 C12N5/10 A61K39/102

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 02557 A (PRAXIS BIOLOG INC) 22 March 1990 (1990-03-22) pages 10,16,17,24; Fig. 17, 20, 24, 25, example 5.4.2. / 5.7.1. / 6.1.2. / 6.8. / 8 / 8.2. ---	1,2,8-17
X	NELSON M B ET AL: "CLONING AND SEQUENCING OF HAEMOPHILUS-INFLUENZAE OUTER MEMBRANE PROTEIN P6" INFECTION AND IMMUNITY, vol. 56, no. 1, 1988, pages 128-134, XP002150261 ISSN: 0019-9567 abstract, page 130, right column; page 131; Fig.1 and 3 page 129, right-hand column --- -/--	1,2,8-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 October 2000	03/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GREEN B A ET AL: "A RECOMBINANT NON-FATTY ACYLATED FORM OF THE HI-PAL P6 PROTEIN OF HAEMOPHILUS-INFLUENZAE ELICITS BIOLOGICALLY ACTIVE ANTIBODY AGAINST BOTH NONTYPEABLE AND TYPE B HAEMOPHILUS-INFLUENZAE" INFECTION AND IMMUNITY, vol. 58, no. 10, 1990, pages 3272-3278, XP000952743 ISSN: 0019-9567 cited in the application the whole document ---	
A	YANG Y -P ET AL: "Effect of lipid modification on the physicochemical, structural, antigenic and immunoprotective properties of Haemophilus influenzae outer membrane protein P6" VACCINE,GB,BUTTERWORTH SCIENTIFIC. GUILDFORD, vol. 15, no. 9, 1 June 1997 (1997-06-01), pages 976-987, XP004115363 ISSN: 0264-410X cited in the application the whole document -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/17020

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 16 and 17 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/17020

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9002557 A	22-03-1990	US 5098997 A	24-03-1992
		AT 124420 T	15-07-1995
		AU 651030 B	07-07-1994
		AU 3379693 A	29-04-1993
		AU 631378 B	26-11-1992
		AU 4228889 A	02-04-1990
		DE 68923286 D	03-08-1995
		DE 68923286 T	07-03-1996
		DK 35891 A	30-04-1991
		EP 0432220 A	19-06-1991
		JP 4502147 T	16-04-1992
		KR 162488 B	16-11-1998
		KR 170752 B	01-10-1999
		US 5196338 A	23-03-1993

PA NT COOPERATION TREAT

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 March 2001 (07.03.01)	
International application No. PCT/US00/17020	**Applicant's or agent's file reference** 33484-00/PCT
International filing date (day/month/year) 20 June 2000 (20.06.00)	**Priority date** (day/month/year) 25 June 1999 (25.06.99)
Applicant METCALF, Benjamin, J.	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 January 2001 (15.01.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer C. Cupello
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 January 2001 (04.01.2001)

PCT

(10) International Publication Number
WO 01/00790 A1

(51) International Patent Classification[7]: C12N 1/21, 15/70, 5/10, A61K 39/102

(21) International Application Number: PCT/US00/17020

(22) International Filing Date: 20 June 2000 (20.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/141,061 25 June 1999 (25.06.1999) US

(71) Applicant *(for all designated States except US)*: AMERICAN CYANAMID COMPANY [US/US]; Five Giralda Farms, Madison, NJ 07940 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: METCALF, Benjamin, J. [US/US]; 15 Rensselaer Drive, Rochester, NY 14618 (US).

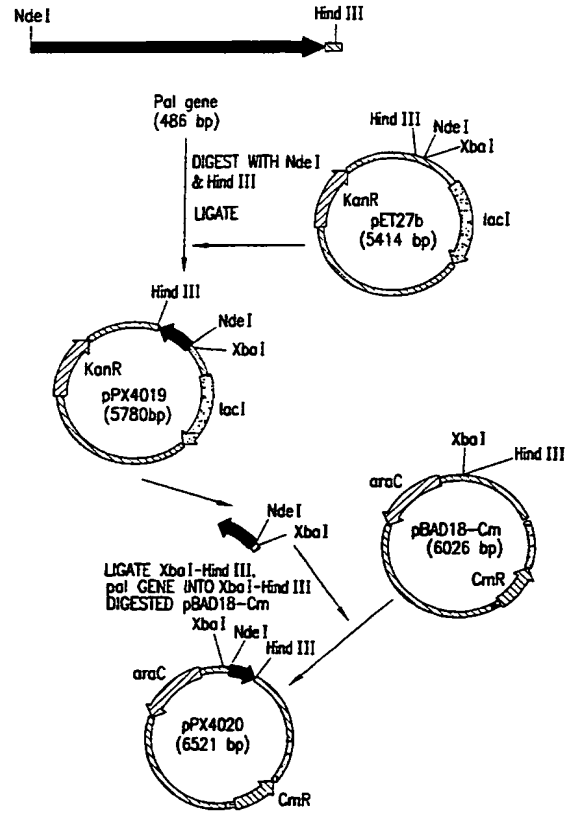
(74) Agents: GORDON, Alan, M.; American Home Products Corporation, Patent Law Department- 2B2, One Campus Drive, Parsippany, NJ 07054 et al. (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian

[Continued on next page]

(54) Title: PRODUCTION OF THE LIPIDATED FORM OF THE PEPTIDOGLYCAN-ASSOCIATED LIPOPROTEINS OF GRAM-NEGATIVE BACTERIA



(57) Abstract: The expression of the lipidated form of the peptidoglycan-associated protein (PAL) of gram-negative bacteria is achieved through the use of a plasmid containing a tightly regulated promoter. A bacterial host cell is transformed, transduced or transfected with such a plasmid. The host cell is then cultured under conditions such that the lipidated recombinant PAL is expressed. The lipidated recombinant PAL is included in an antigenic composition administered to a mammalian host to immunize against a gram-negative bacterium.

WO 01/00790 A1

patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *With international search report.*

— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PATENT COOPERATION TREATY

PCT

REC'D 20 SEP 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
33484-00/PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/17020	20/06/2000	25/06/1999
International Patent Classification (IPC) or national classification and IPC C12N1/21		
Applicant AMERICAN CYANAMID COMPANY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/01/2001	18.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Loubradou, G Telephone No. +49 89 2399 8543

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/17020

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-40 as originally filed

Claims, No.:

1-17 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

Sequence listing part of the description, pages:

1, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-7
	No:	Claims	1, 2, 8-17
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-17
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: WO 90 02557 A (PRAXIS BIOLOG INC) 22 March 1990 (1990-03-22)

D2: YANG Y -P ET AL: 'Effect of lipid modification on the physicochemical, structural, antigenic and immunoprotective properties of Haemophilus influenzae outer membrane protein P6' VACCINE,GB,BUTTERWORTH SCIENTIFIC. GUILDFORD, vol. 15, no. 9, 1 June 1997 (1997-06-01), pages 976-987, XP004115363 ISSN: 0264-410X cited in the application

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. D1 discloses a plasmid containing the lac promoter wherein said promoter is operatively linked to an isolated DNA sequence encoding the PBOMP-1 protein (PBOMP-1 is an other name for the P6 protein of the present application) (see page 81 lines 3 to 22). The possibility to use alternative regulated promoter is disclosed page 29 line 27 to page 30 line 23. The protein obtained using said construct is inherently in the lipidated form since it contains all the signals necessary for the lipidation of the protein.
   Said plasmid was expressed in several E. coli strains including for example JM103 and HB101 strains (page 81 lines 23 to 25). Said strains were cultured in conditions which permit the expression of the lipidated recombinant PBOMP-1 protein.
   Therefore D1 is prejudicial to the novelty of claims 1, 2 and 8 to 10.
   The attention of the applicant is drawn to the fact that the use of the vague and unclear terms "tightly regulated promoter" leaves the reader in doubt as to the meaning of the technical features to which they refer. Such terms cannot be used to delimit the scope of claim 1 from the prior art (see also paragraph 4).

2.1 The attention of the applicant is drawn to the fact that a product is not rendered novel merely by the fact that it is produced by the way of a new process. Since, the lipidated recombinant P6 protein of the present application was not shown to

be different or to have different properties when compared to lipidated P6 purified from H. influenzae, antigenic compositions prepared with lipidated P6 purified from H. influenzae are considered prejudicial to the novelty of claims 11 to 13. There is many examples of such compositions in the prior art, see for example D2 (paragraph bridging pages 978 and 979, second full paragraph of the right hand column of page 979 and paragraph bridging pages 982-984). In said example aluminium phosphate is used is some cases as an adjuvant and several mammals (guinea pigs, mice and rabbits) were immunized. Therefore, D2 is prejudicial to the novelty of claims 11 to 17.

2.2 In addition, recombinant lipidated P6 protein are also known from the prior art. The production of recombinant lipidated P6 protein is disclosed in D1 section 6.8 pages 70 and 71. D1 also discloses the use of adjuvants including aluminium hydroxide and aluminium phosphate (see page 39 lines 18 to 33) and the immunisation of mammals (see page 37 lines 26 to 35 and page 38 lines 15 to 21). Therefore, D1 is also prejudicial to the novelty of claim 11 to 17.

3. The subject-matter of claims 3 to 7 appears to be novel with respect to the prior art cited in the International Search Report. The cited documents do not mention or suggest the use of a more "tightly regulated promoter" in order to solve the problem due to the instability of the lipidated P6 protein.
However, it is well known in the art of recombinant protein production that in the case of unstable protein inducible promoters with tight regulation should be used. In addition, the present application does not provide comparative examples with the plasmids of the closest prior art (see paragraphs 1. and 2. of the present Written Opinion) . Moreover, it is at present not clear whether the plasmid of the present application itself would be responsible for the increased amount of protein produced or whether the combination of specific plasmid/host cell is required (see page 7 line 30 to page 8 line 2). Therefore, at present claims 3 to 7 are not considered as involving an inventive step (Article 33.3 PCT).

4. Claims 16 and 17 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item VIII

Certain observations on the international application

5. As already mentioned in paragraph 1., the terms "tightly regulated promoter" used in claim 1 are vague and unclear and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT). This objection is of major relevance since the choice of the promoter appear to correspond to the core of the present application and since a prior art document with very close subject-matter, D1, exists.

6. The plasmid of claim 1 should not be defined in term of the function to be achieved, "wherein said DNA sequence, under the control of said promoter is expressed in lipidated form" (the applicant should also note that a DNA sequence cannot be expressed in lipidated form, only the protein encoded by said DNA can be expressed in lipidated form) (Article 6 PCT).
The same is also true for the antigenic composition of claim 11 "wherein said antigenic composition elicits a protective immune response in a mammalian host".

7. Unless the designation for a plasmid is internationally accepted (e.g. pUC18), a plasmid should not be identified in a claim by the use of an arbitrary designation. The plasmids "pPX4020" and "pPX4019" of claims 5 and 7 should therefore be characterised by technical features. Alternatively a deposit number could be given in the claims if such a basis exist in the application as originally filed.

8. The attention of the applicant is drawn to the fact that although no unity objections were at present raised (due to the lack of novelty of claims 11 to 17), there is no "special technical features" in the sense of Article 13.2 PCT linking the subject-matter of claims 1 to 10 and the subject matter of claims 13 to 17 since the subject-matter of claims 11 and 12 which formally links the two inventions, is clearly not novel (see paragraph 2.2).